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Thirty-Eight Blood, Tissue and Consumptive Measures from Rats Exposed Perinatally and as Adults to 0.5 Hz Magnetic Fields

by
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ABSTRACT. — In a factorial design, 40 male rats 195 ± 15 days of age that had been exposed: (1) perinatally for 5 days to either 10^{-6} T to 10^{-3} T variation, 0.5 Hz rotating magnetic fields (RMFs) or to sham field conditions, and (2) to one of two typical post-weaning caging conditions were exposed for 5 days to either 10^{-6} T, 10^{-7} T, 10^{-8} T, 0.5 Hz RMFs or sham fields ($<10^{-9}$ T variation in an experimental room) or to colony room control conditions. Consumptive behaviors, selected tissue weights, differential white blood cell counts and 20 constituents from blood sera (analyzed by sequential multiple analyses computer) were measured. Except for marginally significant differences in Na, Cl, and Ca, there were no significant differences between adult RMF intensities for any of the measures; a significant caging by adult intensity interaction was noted for GOT. Rats that had been exposed to the experimental room (continuous noise and light) displayed significant decreases in serum albumin, globulin, glucose and phosphorus levels as well as food and water consumption. Rats that had been exposed perinatally to the RMFs displayed significant 20% reductions in UREA, GOT, and LDH activity, 4% increases in testicle weights and 17% decreases in thymus weights relative to perinatal sham field controls. The absence of significant perinatal condition by adult condition interactions did not support the hypothesis that perinatal RMF exposure might enhance responsiveness to more natural, less intense field variations. Caging differences were associated with highly significant alterations in body weight, several tissue weights, Na, Ca, protein, cholesterol, triglycerides, glucose, CO_2 and LDH.

INTRODUCTION

The possible effects of ELF (extremely low frequency) or biofrequency electromagnetic fields upon life systems have interesting practical and theoretical implications (Persinger, 1974; Persinger, Ludwig and Ossenkopp, 1973). A variety of different frequencies (0.1 to 100 Hz), variation intensities (10^{-5} T to 10^{-2} T or 0.1 to 100 G; 10^{1} V/m to 10^{3} V/m) and wave patterns (sine waves and μs pulses) have been associated with significant organismic changes. This laboratory has been involved with the investigation of the effects and possible mechanisms of 0.5 Hz rotating magnetic fields (RMFs) for over a decade; for a review, see (Persinger, Lafrenière and Ossenkopp, 1974). The RMF,

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produced by rotating permanent magnets by a series of pulleys and shafts turned by a motor, is an inexpensive and reliable means to produce time-varying magnetic fields for long periods of time. Intensity levels can be easily controlled by varying the exposure distance from the magnets or by using different sets of magnets.

Rats exposee to these fields during various prenatal and postnatal periods of development have demonstrated different patterns of replicable and significant changes in emotional responses (Persinger, 1969; Persinger and Foster, 1970; Persinger and Pear, 1972), consumptive behaviours, and certain endocrine tissues (Persinger, Lafrenière and Ossenkopp, 1974). Most of these studies involved 0.5 Hz variation intensities between 10^{-5}T to 10^{-2}T. In the pursuit of mechanism, we decided to determine if: (1) long term modifications in blood constituents and tissue weights occurred in rats that had been exposed perinatally to 0.5 Hz magnetic field intensities previously associated with behavioural effects and (2) re-exposure of these rats to less intense, 10^{-6} T to 10^{-8}T, 0.5 Hz magnetic fields would demonstrate enhanced organismic sensitivity to these fields. Adult exposure variation intensities of 10^{-6}T to 10^{-8}T were chosen since many man-made and geomagnetic ELF magnetic field patterns occur within this range. Although certain theoretical notions would contend no effect below 10^{-4}T variation, this is the first study actually: (1) to measure blood profiles in rats exposed to such low intensities, and (2) evaluate possible perinatal-adult magnetic field exposure interactions.

The detection of any statistically significant organismic changes from such low intensities would have to be evaluated carefully. Statistical significance does not necessarily determine practical significance (a point forgotten by some enthusiastic researchers) and may only reflect the sophistication of the measurement procedure. Secondly, the degree of change should be compared to changes from other variables incorporated within the experimental design such that some reference can be used for the absolute magnitude of the magnetic effect. In this study, the comparator variable was postweaning caging conditions: plastic cages containing bedding vs wire rack caging.

METHOD

SUBJECTS. — Forty (40) 180 to 210 day old naive male albino Wistar rats selected from 18 different litters that had been exposed perinatally for 5 days (—2.5 days to +2.5 days of age) to either: (1) 10^{-3}T, 10^{-4}T or 5 x 10^{-6}T variation 0.5 Hz magnetic fields, or (2) to one of three sham-field control conditions, were used as subjects. The mothers of the subjects had been obtained from Bio Breeding Laboratories of Canada Limited.

APPARATUS. — The rotating magnetic field (RMF) was produced by rotating two horseshoe magnets about their major axes by an electric motor at 30 RPMs. The intensity of the 0.5 Hz field could be controlled by: (1) varying the exposure distances from the magnets, and (2) using one of three different sets of magnets. As an example of intensity time-variation, field intensity changes within the 1 G variation exposure area varied from 0.5 G N polarity to 0.5 G S polarity (i.e., 1 G "peak to peak") and then back to 0.5 G N polarity every 2 s (0.5 Hz). A gradual movement in exposure space of the point where the two most intense fields of two magnets interacted was associated with a maximum 0.5 Hz variation to about 1/5 that variation intensity every 30 to 40 min. The latter sine wave-like rhythm was mechanically produced by a slight difference in pully belt sizes for the two magnet shafts. Such patterns of 30 to 40 min "trains" of 0.5 Hz amplitudes were considered desirable since they closely simulated some forms of geomagnetic variations. Exposure areas were supported securely and independently from the main plywood base to which the motor was attached. An on-line electric clock

served as a constant check against possible power failures. Lighting was indirect and continuous from overhead fluorescent lamps and was measured by a Gossen electronic Panlux meter to be 45±5 lx within the cages of all exposure areas. The motor, which was operative in all conditions, produced a continuous background sound of 70 db as determined by a Type 2203 B and K Soundlevel meter. Temperature was maintained by a homeostat at 24.5 ± 0.2° C. The static geomagnetic-cultural field in the exposure areas with all magnets removed was measured to be 80,000 ± 2000 γ (vertical component) by a McPhar magnetometer.

PERINATAL EXPOSURE. — Three exposure variation intensities: 10^{-3} T, 10^{-4} T and 5 x 10^{-6} T, produced by horseshoe magnets attached to the output shafts and known to be associated with later behavioural and physiological changes in rats were used. The sham field conditions involved the normally operating apparatus with the magnets removed. Previous measurements have indicated no significant differences in either noise or vibration between the magnet-on and magnet-off condition.

ADULT EXPOSURE. — Two sets of specially constructed magnets equal in weight to the nearest g were used. The first set was composed of iron and produced 0.5 Hz variation intensities of 2000 γ (2 x 10^{-6} T) and 100 γ within the exposure areas. A second set of "magnets" were constructed almost entirely of aluminum and were weighted with plaster. However when attached to the output shafts, a residual magnetization induced weak 0.5 Hz variation fields of 10 γ and <1 γ (adult sham field) in the exposure areas as determined by a magnetometer. Since the 2000 γ and 10 γ areas and the 100 γ and <1 γ areas were the same spatial positions in the RMF apparatus, respectively, a built-in control was established to detect any possible position artifacts. The 30 to 40 min rhythm in maximum-minimum 0.5 Hz variation was measured also within all of these exposure positions.

PROCEDURE

PERINATAL AND POSTWEANING TREATMENTS. — Between 25 October and 15 December 1975, a total of 18 pregnant female rats were exposed to either RMF or perinatal sham field conditions. Nine females were exposed in three separate experimental series (3/series) to one of three RMF variation intensity areas: 10^{-3} T, 10^{-4} T or 5 x 10^{-6} T, previously described. Another 9 females were exposed in three separate experimental series to the three sham-field conditions which involved the same areas as the RMF exposures only the magnets had been removed. Females were exposed to the experimental situations (RMF or sham) on day 19 of gestation, about 2.5 days before birth. All females were housed in 28 x 18 x 12 cm (deep) plastic cages containing 0.6 mm corncob (Bedocob), surrounded by white cardboard and covered with a wire top. Purina rat chow and tap water were available ad libitum; water was changed daily. Within 24 h of birth, each litter was temporarily removed (about 5 min) from the experimental situation and reduced to 8 pups. About 2.5 days after birth, mother and pups were removed from the experimental situation, returned to the colony room, and placed in 46 x 20 x 14 cm plastic cages containing 200 ml of corncob. The bedding was changed on the 11th and 16th postnatal days only. At 21 days of age, the pups were weaned and earpunched for identification.

Male rats from the same or different litters were housed (3/cage) permanently in either: (1) standard wire 40.5 x 25 x 18 cm (deep) cages or (2) 46 x 20 x 14 cm (deep) plastic cages containing Bedocob that was changed biweekly. The positions of the cages containing RMF-exposed or sham-field rats were alternated within the rows of both racks and plastic cages. Food and water were available ad libitum. Temperature within the colony room was 22 ± 1° C and the sound level 70 ± 2 db; it was produced primarily by the continuous operation of automatic cages (not used in the study). A LD 12:12 cycle was maintained with light onset beginning 09:00 h.

ADULT EXPOSURE TREATMENTS. — Eight separate experimental series were completed between 9 May and 27 June 1976. The series order using the aluminum magnets (AM) and the iron magnets (IM) was: IM, AM, IM, AM, AM, IM, AM, IM. Animals that had been housed in the plastic cages were used in series 1, 2, 7 and 8 while those that had been housed in the standard wire cages were used in series 3 to 6. In each run, 5 rats were housed singly in the same type cages used for the pregnant females. Four of the rats (pairs of perinatally RMF-exposed and sham-exposed litter mates) were taken to the experimental room used for the perinatal exposures and exposed to one of the two RMF (IM or AM) conditions for 5 days. One litter mate from each pair was placed such that each intensity level always had one perinatal sham-field and one perinatal RMF-exposed subject. The three perinatal RMF or sham conditions were counterbalanced within the adult conditions. The other rat of each series remained in the colony room as adult colony controls; equal numbers of perinatal sham field and perinatal RMF rats were used. Purina rat chow and water delivered in 100 ml tubes with 1 ml graduations were available ad libitum. Fresh water was given daily at which time the amount consumed during the previous 24 h was measured. The total food consumed during the exposure period was determined at the end of the experiment by subtracting the weight of the remaining food blocks from the original amount placed in the cage.

SERUM COLLECTION AND ANALYSES. — After 5 days of exposure to the experimental or colony situations, the subjects were removed separately to the laboratory and decapitated; this process required about 5 min. Blood was collected and allowed to stand for 15 min after which it was centrifuged at 3400 rpm for 10 min. The serum was removed, centrifuged again, placed in coded test tubes, refrigerated overnight at 4°C and analyzed on a Technicon SMAC System (calibrated daily) by the technical group at Laurentian Hospital for: albumin, protein, glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, carbon dioxide, uric acid, albumin/globulin ratios (A/G), calcium, phosphorous, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), SGOT (serum glutamate oxaloacetic transaminase), SGPT (serum glutamate pyruvate transaminase), bilirubin (total), cholesterol and triglycerides.

TISSUE AND LEUKOCYTE MEASUREMENTS. — During the first, middle and last portion of the 45 to 50 s sequence of major blood flow following decapitation, single drops were collected and smeared on microscope slides; when dried they were processed with Wright's stain for leukocytes. Adrenals, thymuses, spleens and testicles were weighed to the nearest 0.1 mg on a Mettler analytical balanced by G.F.L. The best two smears of the three slides collected for each animal was observed by N.J.C. under oil immersion at 1000 X to determine leukograms. The average of 2 to 4 separate fields of 100 cells per slide was used to determine the leukocyte profile of each animal. Three way analyses of variance (ANOVAs) for 5 adult exposure intensities (3 fields, +1 sham field, +1 colony control), two perinatal conditions (RMF-exposed vs sham field exposed) and two postweaning caging conditions (standard wire vs plastic cages) as well as analyses of covariance, means, standard deviations, a posteriori tests and analyses of variance to detect possible artifacts were completed by IBM 360-40 computer using SPSS programs; sample analyses were checked manually.

RESULTS

BLOOD SERUM CONSTITUENTS

The means and standard deviations for the various blood serum measures according to: adult intensity, perinatal condition and postweaning caging are shown in Tables 1 and 2. The only significant ($P < 0.05$) interaction (Fig. 1) was between adult intensity and caging for GOT ($F = 2.83$; $df = 4, 20$). Significant differences between adult con-

ditions (df = 4, 20) were apparent for sodium ($F = 5.55$; $P<0.01$), chloride ($F = 3.10$; $P = 0.05$), phosphorus ($F = 5.29$; $P<0.01$), calcium ($F = 3.00$; $P<0.05$), A/G ratios ($F = 3.51$; $P<0.05$) and glucose ($F = 4.36$; $P = 0.01$). However a posteriori tests (Scheffe's and Duncan's set at $P<0.05$) indicated that only (1) sodium, chloride and

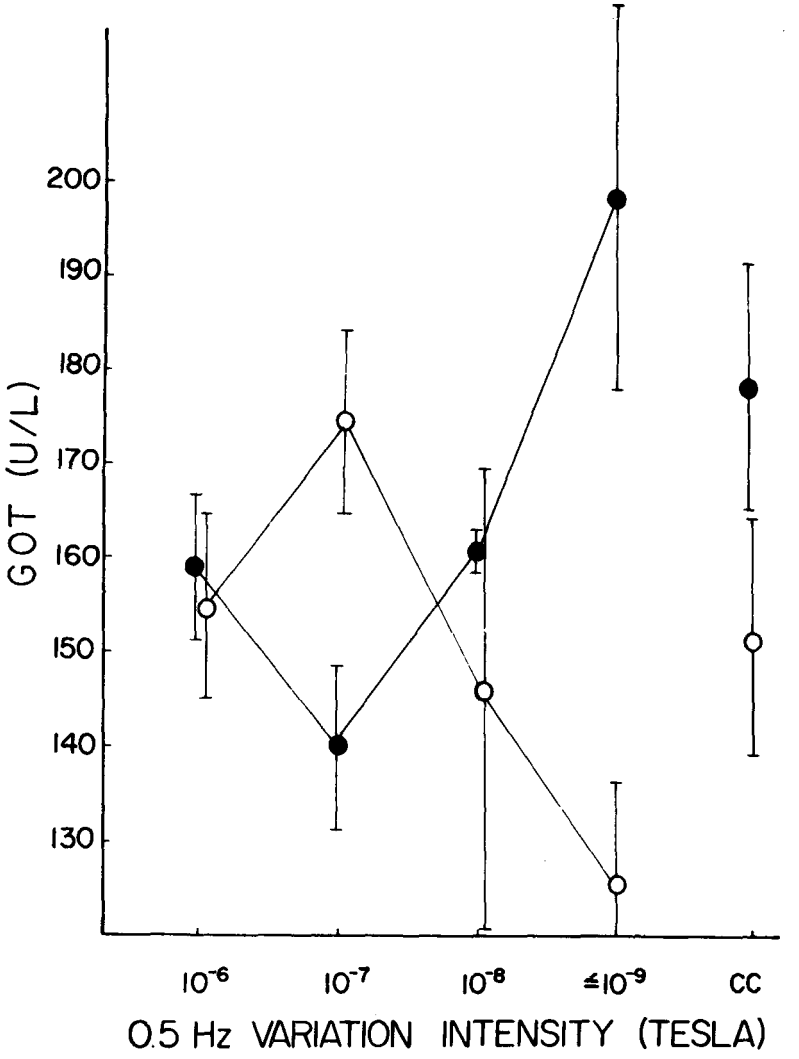

Fig. 1. Means and standard deviations (vertical bars) in GOT activity in blood sera from rats that had been exposed for 5 days to different 0.5 Hz magnetic field intensities (in Tesla), sham field (10^{-9}T), or colony control (CC) conditions. Pre-exposure, postweaning caging conditions were: plastic cages (closed circles) and wire cages (open circles); see text for cage descriptions. Each circle represents 4 rats.

Table 1. Means and standard deviations (±) for 20 blood serum measures from rats according to main adult exposure conditions: 3, 0.5 Hz magnetic field intensities (in Tesla), sham field and colony room control.

	FIELD ROOM				COLONY ROOM
	Sham Field (n = 8)	10^{-8}T (n = 8)	10^{-7}T (n = 8)	10^{-6}T (n = 8)	Colony Controls (n = 8)
Albumin (g/dl)	5.0 ± 0.4	4.9 ± 0.4	5.0 ± 0.4	4.9 ± 0.4	5.3 ± 0.3
Protein (g/dl)	7.9 ± 0.3	7.9 ± 0.5	8.2 ± 0.3	8.0 ± 0.4	7.9 ± 0.4
Glucose (mg/dl) x 10	13.5 ± 1.5[a]	13.8 ± 1.4[a]	14.1 ± 0.7[a]	14.0 ± 0.8[a]	15.9 ± 1.0[b]
Urea (mg/dl)	19.8 ± 2.7	19.8 ± 3.4	20.3 ± 2.3	21.0 ± 2.3	19.4 ± 1.4
Creatinine (mg/dl)	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Na (meq/l) x 10	14.4 ± 0.2	14.3 ± 0.3[a]	14.5 ± 0.1[b]	14.4 ± 0.2	14.6 ± 0.2[b]
K (meq/l)	7.0 ± 0.3	7.1 ± 0.5	6.9 ± 0.3	7.0 ± 0.4	7.0 ± 0.4
Cl (meq/l x 10	10.3 ± 0.2	10.1 ± 0.1[a]	10.2 ± 0.1[b]	10.1 ± 0.2[a]	10.3 ± 0.2[b]
CO_2 (meq/l)	27.9 ± 3.4	28.4 ± 2.7	28.6 ± 1.7	28.6 ± 1.4	29.3 ± 3.0
Uric A (mg/dl)	9.8 ± 1.8	10.6 ± 2.6	10.6 ± 1.4	10.5 ± 2.4	10.6 ± 0.7
A/G	1.8 ± 0.4	1.7 ± 0.3[a]	1.6 ± 0.3[a]	1.6 ± 0.2	2.1 ± 0.2[c]
Ca (mg/dl)	10.7 ± 0.3[a]	10.7 ± 0.6[a]	11.3 ± 0.4[b]	11.1 ± 0.4	11.0 ± 0.6
P (mg/dl)	6.5 ± 0.5'	6.2 ± 0.2[a]	6.5 ± 0.4	6.1 ± 0.5[a]	7.0 ± 0.6[b]
LDH (U/l) x 10^2	15.8 ± 4.2	15.2 ± 4.7	15.4 ± 2.7	16.0 ± 3.0	16.2 ± 3.0
ALP (U/l) x 10^1	17.4 ± 5.0	18.1 ± 4.1	19.1 ± 2.9	19.4 ± 3.0	18.2 ± 3.8
GOT (U/l) x 10^1	16.2 ± 5.7	15.4 ± 4.0	15.8 ± 3.5	15.7 ± 2.5	16.1 ± 2.1
GTP (U/l) x 10^1	5.3 ± 1.1	6.2 ± 1.5	5.2 ± 1.5	5.3 ± 1.1	5.5 ± 0.6
Bilirubin (mg/dl)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Cholesterol (mg/dl) x 10^1	11.2 ± 1.7	11.5 ± 2.2	11.9 ± 1.8	12.2 ± 2.9	11.9 ± 1.7
Triglycerides (mg/dl) x 10^1	18.6 ± 9.8	19.1 ± 9.9	20.4 ± 8.2	23.8 ± 7.7	22.8 ± 9.2

a vs b = $P < 0.05$
a vs c = $P < 0.01$

Table 2. Means and standard deviations (±) for 20 blood serum measures for the 40 rats according to perinatal exposure condition: RMF (0.5 Hz rotating magnetic field) vs sham-field, or to postweaning, pre-exposure (adult) caging conditions: plastic (P) vs wire (W).

	PERINATAL CONDITION		POSTWEANING CAGING	
	Sham Field (n = 20)	RMF (n = 20)	W (n = 20)	P (n = 20)
Albumin (g/dl)	5.0 ± 0.3	5.0 ± 0.5	4.9 ± 0.2	5.1 ± 0.5
Protein (g/dl)	8.0 ± 0.3	8.0 ± 0.4	7.7 ± 0.3[a]	8.2 ± 0.2[d]
Glucose (mg/dl) x 10^1	14.3 ± 1.5	14.3 ± 1.5	13.8 ± 0.8[a]	14.6 ± 1.6[b]
Urea N (mg/dl)	21.1 ± 2.3[a]	19.0 ± 2.1[c]	20.4 ± 3.0	19.7 ± 1.7
Creatinine (mg/dl)	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
Na (meq/l) x 10^1	14.4 ± 0.2	14.4 ± 0.2	14.3 ± 0.2[a]	14.6 ± 0.1[d]
K (meq/l) x 10^1	7.0 ± 0.4	7.0 ± 0.4	6.9 ± 0.4	7.1 ± 0.4
Cl (meq/l) x 10^1	10.2 ± 0.2	10.2 ± 0.2	10.1 ± 0.2	10.2 ± 0.2
CO_2 (meq/l)	28.3 ± 2.3	28.8 ± 2.4	29.5 ± 2.3[a]	27.7 ± 2.2[b]
Uric A (mg/dl)	10.5 ± 1.7	10.4 ± 2.0	10.2 ± 2.0	10.7 ± 1.7
A/G	1.8 ± 0.3	1.7 ± 0.4	1.8 ± 0.3	1.7 ± 0.4
Ca (mg/dl)	10.8 ± 0.5[a]	11.1 ± 0.5[b]	10.7 ± 0.5[a]	11.2 ± 0.4[c]
P (mg/dl)	6.4 ± 0.6	6.4 ± 0.4	6.5 ± 0.4	6.4 ± 0.7
LDH (U/l) x 10^2	17.1 ± 3.5[a]	14.4 ± 2.8[b]	16.8 ± 3.1	14.6 ± 3.5
ALP (U/l) x 10^1	18.9 ± 3.8	17.9 ± 3.6	17.3 ± 3.5	19.6 ± 3.7
GOT (U/l) x 10^1	17.3 ± 3.8[a]	14.5 ± 2.9[c]	16.6 ± 3.2	15.2 ± 3.9
GPT (U/l) x 10^1	5.7 ± 1.0	5.3 ± 1.4	5.9 ± 1.2	5.1 ± 1.1
Bilirubin (mg/dl)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
Cholesterol (mg/dl) x 10^1	11.6 ± 1.9	11.8 ± 2.2	10.3 ± 1.0[a]	13.2 ± 1.8[d]
Triglyceride (mg/dl) x 10^1	21.5 ± 9.3	20.4 ± 9.5	16.8 ± 7.5[a]	25.1 ± 9.2[c]

a vs b = $P < 0.05$
a vs c = $P < 0.01$
a vs d = $P < 0.001$

calcium levels demonstrated significant differences between variation intensities and (2) only the calcium changes were consistent in all experiments; the sodium and chloride significances were primarily due to the influence of only four experiments. Major relative differences occurred between the 10^{-8}T and 10^{-1}T groups. The differences in the other changes were primarily between the colony controls and the rats exposed to the experimental room that was characterized by continuous lighting and background sound.

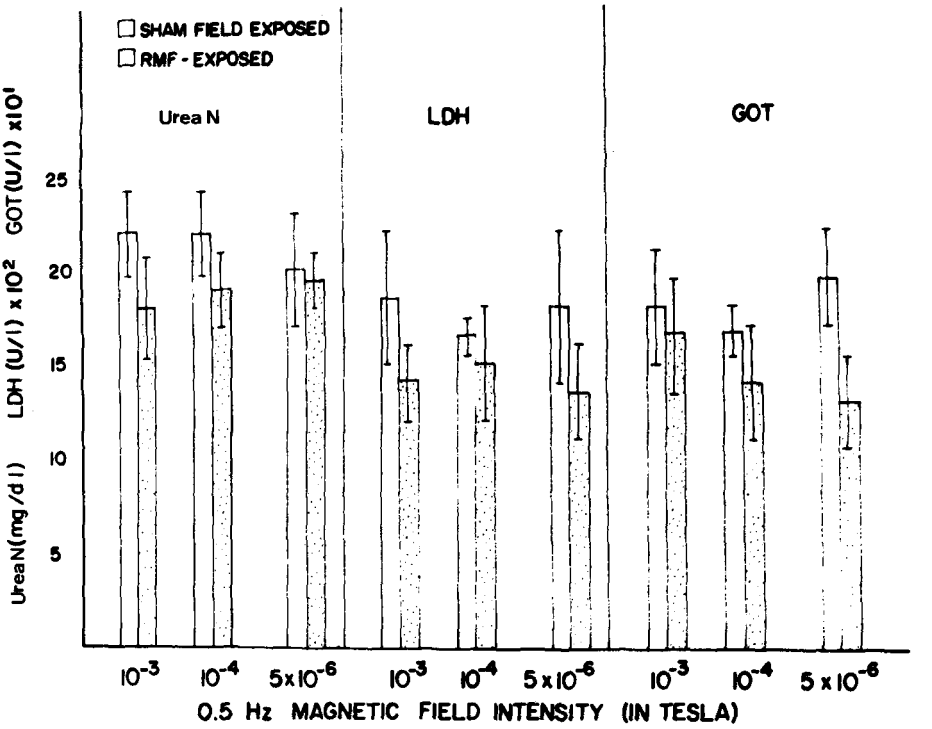

Fig. 2. Means of blood urea nitrogen, GOT activity and LDH activity from 180- to 210-day-old rats that had been exposed for five days around birth to different intensity 0.5 Hz magnetic fields or to the same positions (sham fields) in the absence of 0.5 Hz magnetic fields. Vertical bars indicate standard deviations.

Rats that had been exposed perinatally to the RMF displayed significant reductions (Table 2) in urea N ($F = 9.01$; $P < 0.01$), LDH ($F = 6.06$; $P < 0.05$) and GOT ($F = 7.41$; $P = 0.01$) relative to sham field exposed rats (df = 1, 20); differences in all other measures were not significant. The means and standard deviations for these measures from two-way ANOVAs (RMF and sham vs exposure positions 1, 2, 3) executed to determine any possible perinatal exposure intensity dependence or position artifacts are shown in Fig. 2. GOT activity demonstrated an intensity-like dependence within the RMF groups, although this trend was not statistically significant ($P > 0.05$). The urea N and LDH were relatively homogeneous across intensity levels. No position artifacts (sham field levels 1, 2, 3) were detected. The urea N, GOT and LDH effects were still

Table 3. Means and standard deviations (±) for absolute and relative wet weights for selected organs, weight loss measures, food and water consumption, and relative blood leukocyte numbers according to the five adult conditions: three field intensities (in Tesla) sham-field, and colony controls.

		0.5 Hz RMF			
	Sham Field	10^{-8}T	10^{-7}T	10^{-6}T	Colony Controls
Adrenals (mg)	21.3 ± 2.1	22.9 ± 2.7	22.0 ± 3.4	20.6 ± 0.9	20.3 ± 2.5
Adrenals (μg/g b.w.)	39.8 ± 4.4	42.8 ± 5.7	39.6 ± 5.5	37.6 ± 3.0	38.4 ± 5.3
Thymus (g)	0.36 ± 0.1	0.33 ± 0.11	0.33 ± 0.08	0.38 ± 0.1	0.36 ± 0.07
Thymus (μg/g b.w.) x 10^2	6.61 ± 1.73	6.01 ± 1.87	5.98 ± 1.46	6.92 ± 1.48	6.82 ± 1.3
Spleen (g)	0.88 ± 0.17	0.88 ± 0.14	0.88 ± 0.08	0.93 ± 0.16	0.87 ± 0.1
Spleen (mg/g b.w.)	1.62 ± 0.23	1.64 ± 0.2	1.60 ± 0.13	1.69 ± 0.25	1.65 ± 0.14
Testicles (g)	3.84 ± 0.28	3.92 ± 0.28	3.96 ± 0.17	4.13 ± 0.30	3.88 ± 0.23
Testicles (mg/g b.w.)	7.13 ± 0.54	7.30 ± 0.62	7.27 ± 0.60	7.54 ± 0.56	7.25 ± 0.54
Body weight (g)	539.0 ± 36.0	539.0 ± 54.0	544.0 ± 51.0	550.0 ± 32.0	540.0 ± 50.0
Weight loss (g)	−3.80 ± 21.8	−4.10 ± 19.3	11.00 ± 14.6	−1.4 ± 9.0	−2.4 ± 7.8
Weight loss (%)	−0.61 ± 3.9	−0.76 ± 3.6	−1.85 ± 2.4	−0.2 ± 1.6	−0.3 ± 1.3
H_2O (ml/day)	23.6 ± 4.7[a]	27.5 ± 4.8	24.4 ± 5.9[a]	24.0 ± 5.0[a]	31.3 ± 4.6[b]
H_2O (ml/(100 g b.w. d))	4.48 ± 0.80	5.05 ± 0.71	4.59 ± 1.39	4.36 ± 0.76	5.84 ± 0.71
Food (g/5 day)	92.0 ± 26.0	110.0 ± 26.0	99.0 ± 22.0	102.0 ± 14.0	114.0 ± 13.0
Food (g/(100 g b.w. 5d))	16.9 ± 4.4	20.2 ± 3.7	17.8 ± 4.0	18.6 ± 2.8	21.5 ± 1.7
Lymphocytes (%)	78.8 ± 5.2	77.8 ± 5.2	81.5 ± 4.2	80.3 ± 5.0	83.1 ± 5.1
Neutrophils (%)	20.6 ± 5.5	21.1 ± 5.0	17.5 ± 4.4	18.7 ± 4.8	15.6 ± 5.0
Eosinophils (%)	0.53 ± 0.35	0.94 ± 0.74	0.90 ± 0.5	0.93 ± 0.54	1.44 ± 0.85

a vs b = $P < 0.05$

significant ($P < 0.05$) even after covariance for body weight or experiment number were performed before the analyses of variance.

The largest number of significant differences occurred between cage conditions (df = 1, 20). The 20 rats that had been exposed to the plastic cage condition (since weaning) before the adult series demonstrated (Table 2) elevated serum sodium ($F = 35.58$; $P < 0.001$), calcium ($F = 11.70$; $P < 0.01$), protein ($F = 36.01$; $P < 0.001$), cholesterol ($F = 32.85$; $P < 0.001$), triglycerides ($F = 9.36$; $P < 0.01$) and glucose ($F = 4.67$; $P < 0.05$) levels but decreased carbon dioxide ($F = 4.84$; $P < 0.05$) and LDH ($F = 4.00$; $P < 0.05$) levels, relative to rats maintained in wire cages.

TISSUE, CONSUMPTIVE AND LEUKOCYTE MEASURES

The means and standard deviations for the various tissue wet weights (absolute and relative to body weight), body weight loss (absolute and %), daily water consumption (absolute and per 100 g body weight), total food consumption (absolute and per 100 g body weight) and white blood cell (lymphocytes, neutrophils and eosinophils; other cell types were too infrequent to be statistically valid) percentages according to the main effects: 5 adult conditions, two perinatal conditions, and two caging conditions, are shown in Tables 3 and 4. Three way analyses of variance demonstrated no statistically significant interactions. Significant differences between adult conditions were noted for absolute water consumption ($F = 3.12$; df = 4, 20; $P < 0.05$) only; relative water consumption was not significant but in the same direction ($F = 2.37$; $P < 0.10$). A posteriori tests (Duncan's and Scheffe's set at $P < 0.05$) indicated that the primary difference existed between the colony controls and the rats placed in the experimental room, regardless of RMF intensity. The 20 rats that had been exposed perinatally to the RMFs displayed significantly lighter absolute ($F = 4.78$; df = 1, 20; $P < 0.05$) and relative ($F = 6.91$; $P = 0.01$) thymus weights and heavier absolute ($F = 7.88$; $P = 0.01$) and relative ($F = 4.38$; $P < 0.05$) testicle weights relative to the perinatal sham field rats. Separate analyses for perinatal intensity conditions demonstrated some field intensity dependence. Rats that had been exposed perinatally to the 10^{-3}T, 10^{-4}T and 5×10^{-6}T variation RMFs displayed absolute thymus weights of 0.30 ± 0.11, 0.31 ± 0.03, 0.34 ± 0.11 g and absolute testicle weights of 4.03 ± 0.36, 4.08 ± 0.17, and 3.96 ± 0.15 g, respectively while the sham field controls exposed to these positions displayed weights of 0.39 ± 0.12, 0.38 ± 0.06, and 0.39 ± 0.10 and 3.78 ± 0.33, 3.88 ± 0.13 and 3.88 ± 0.33, respectively for these tissues. Further analyses of variance demonstrated neither exposure position artifacts nor differences between sequence of perinatal exposure.

Caging conditions generated the most numerous group differences. Rats that had been maintained in plastic cages before the adult exposures displayed differences in absolute spleen weights ($F = 19.00$, df = 1, 20; $P < 0.001$), relative adrenal weights ($F = 13.95$; $P < 0.001$), absolute testicle weights ($F = 9.51$; $P < 0.01$), relative testicle weights ($F = 14.00$; $P < 0.001$), body weight ($F = 31.93$; $P < 0.001$), absolute water consumption ($F = 7.20$; $P = 0.01$) and absolute food consumption ($F = 6.47$; $P = 0.02$). Analyses of covariance for body weight before analyses of variance indicated that most of the above differences were due to body weight since only relative adrenal weights ($F = 8.59$; $P < 0.01$), absolute spleen ($F = 6.82$; $P < 0.05$), and relative spleen ($F = 4.68$; $P < 0.05$) weights remained significant. However, even after body weight covariance, the absolute thymus ($F = 6.40$, df = 1, 20; $P < 0.05$), relative thymus ($F = 7.38$; $P = 0.01$), absolute testicle ($F = 8.32$; $P < 0.01$) and relative testicle ($F = 7.27$; $P < 0.01$) weight differences between perinatal RMF-exposed and sham field exposed rats remained significant.

DISCUSSION

The results of eight separate sequences involving 5-day exposures of adult rats to sham fields (less than 10^{-9}T) or to 10^{-8}T, 10^{-7}T or 10^{-6}T, 0.5 Hz variation magnetic fields

Table 4. Means and standard deviations (±) for absolute and relative wet weights of selected organs, weight loss measures, food and water consumption and relative blood leukocyte numbers according to the two perinatal conditions: RMF-exposed and sham-field and the two postnatal caging conditions: plastic vs wire.

	PERINATAL CONDITION		CAGING CONDITION	
	Sham Field	RMF	Wire	Plastic
Adrenals (mg)	21.20 ± 3.10	21.60 ± 1.80	21.50 ± 2.40	21.30 ± 2.60
Adrenals (μg/g b.w.)	39.40 ± 5.40	39.90 ± 4.70	42.20 ± 4.20[a]	37.10 ± 4.40[d]
Thymus (g)	0.38 ± 0.09[a]	0.32 ± 0.08[b]	0.33 ± 0.09	0.37 ± 0.09
Thymus (μg/g b.w.) x 10^2	7.09 ± 1.60[a]	5.84 ± 1.22[b]	6.45 ± 1.64	6.49 ± 1.50
Spleen (g)	0.86 ± 0.09	0.92 ± 0.16	0.81 ± 0.07[a]	0.97 ± 0.12[d]
Spleen (mg/g b.w.)	1.61 ± 0.12	1.68 ± 0.24	1.59 ± 0.15	1.69 ± 0.21
Testicles (g)	3.85 ± 0.27[a]	4.04 ± 0.23[c]	3.84 ± 0.25[a]	4.05 ± 0.24[c]
Testicles (mg/g b.w.)	7.15 ± 0.47[a]	7.44 ± 0.60[b]	7.55 ± 0.51[a]	7.04 ± 0.51[d]
Body weight (g)	539.0 ± 40.0	546.0 ± 50.0	509.0 ± 25.0[a]	577.0 ± 31.0[d]
Weight loss (g)	—3.20 ± 14.8	—5.90 ± 15.7	—8.80 ± 14.2	—0.30 ± 15.1
Weight loss (%)	—0.44 ± 2.60	—1.05 ± 2.80	—1.50 ± 2.70	0.00 ± 2.60
H_2O (ml/day)	25.80 ± 5.00	26.50 ± 6.10	24.00 ± 5.10[a]	28.40 ± 5.10[c]
H_2O (ml/(100 g b.w. d))	4.81 ± 0.94	4.91 ± 1.12	4.70 ± 1.02	5.03 ± 1.03
Food (g/5 days)	107.0 ± 19.0	100.0 ± 24.0	95.0 ± 23.0[a]	112.0 ± 16.0[b]
Food (g/(100 g b.w. 5d))	19.80 ± 3.40	18.20 ± 3.80	18.70 ± 4.50	19.30 ± 2.70
Lymphocytes (%)	81.40 ± 5.60	79.20 ± 4.30	80.00 ± 6.10	80.60 ± 3.90
Neutrophils (%)	17.60 ± 5.70	19.80 ± 4.30	18.80 ± 6.20	18.60 ± 3.80
Eosinophils (%)	0.98 ± 0.60	0.92 ± 0.70	1.04 ± 0.70	0.86 ± 0.50

a vs b = $P < 0.05$
a vs c = $P < 0.01$
a vs d = $P < 0.001$

indicate that highly significant shifts in serum profiles do not occur. Such continuous exposure during a 5-day period did not significantly alter adrenal, spleen, thymus, body or testicle weights, although the latter tissue demonstrated a tendency to increase with field intensity; Persinger, Glavin and Ossenkopp (1972) in less controlled studies had reported increased elevations in testicle weights in one of three experiments involving more intense (10^{-3}T), 0.5 Hz RMFs. Differential white blood cell counts also did not differentiate the RMF field exposed rats from the sham field or colony room controls.

Small and marginally significant differences in four blood measures: serum sodium, chloride, calcium and GOT were found between rats exposed as adults to the low intensity fields relative to the sham fields. Since both sodium and chloride measures show less magnitudes at 10^{-8}T and 10^{-6}T (the same exposure positions) than at sham field and 10^{-7}T (also the same exposure positions) the involvement of some unspecified artifact must remain a possible explanation for these changes unless one assumes a complicated intensity relationship. Calcium changes were consistent across experiments and displayed some systematic field intensity-dependence. Both Adey (1975) and Ludwig (1968) have implicated the calcium ion in ELF magnetic field effects, although their models involve nervous tissue.

Serum SGOT levels were also associated with significant field intensity dependent changes, but these effects were influenced differentially by pre-adult exposure caging condition. Elevations of serum GOT have been reported in rodents during exposures to static electric fields of 5 kV/m (Klingenberg et al., 1975) and to 60 Hz electric fields of 150 V/cm (Marino et al., 1977). The latter studies also reported elevations in albumin or neutral fat serum levels, respectively, but such changes were not recorded in these studies. The tendency for elevations in triglycerides around 10^{-6}T variation is interesting in light of the significant elevations in serum lipids following 10-day exposures to 10^{-3}T, 0.5 Hz variation magnetic fields (Persinger, Lafrenière and Ossenkopp, 1974).

The largest RMF-associated effects were displayed by the subjects that had been exposed for 5 days perinatally (more than 190 days before measurement) to 10^{-6}T to 10^{-3}T, 0.5 Hz RMFs, relative to sham field controls; statistical analyses completed after all data had been collected showed significant alterations in thymus weights, testicle weights GOT, LDH and Urea N levels. The testicle results were similar to previous experiments (Ossenkopp, Koltek and Persinger, 1972) where a different strain of rats (Holtzman) were exposed during their entire prenatal development or during the last three days before birth to 0.5 Hz RMFs of comparable intensity. However, the amplitude of the effects are different. Testicle weights were elevated by only 4% (relative to sham field rats) in the present study compared to the 8% to 10% noted previously. Unlike the previous study, thymus weights were significantly lighter for the perinatal RMF-exposed rats. However, there are several procedural differences between the two studies; rats in the 1972 study (Ossenkopp, Koltek and Persinger, 1972) had been maintained at 80% free feed body weight and tested in conditioned suppression (electric shock) schedules daily for 35 to 60 days until two weeks before tissue collection. Since thymus weight is known to change (usually decrease) in rats exposed to excessive stimulation (e.g., electric shock), any RMF-control differences may have been attenuated. Despite the small absolute differences between testicle and thymus weights between perinatal RMF-exposed and sham field-exposed rats, the effects were comparable in magnitude to the differences between types of postweaning caging, a condition sufficient to also significantly alter body weights, consumptive behaviors, and several blood measures.

An unexpected result of the present study was the magnitude of the reduction in serum GOT, LDH and Urea N values of adult rats that had been exposed for five days perinatally to 10^{-6}T to 10^{-3}T RMFs relative to controls, although such exposure intensities have been reported many times to be associated with behavioural-physiological and morphological changes following both perinatal and adult RMF exposures. These RMF-associated enzyme changes were even larger than the effects of either

caging or room conditions. Experiments involving manual GOT measurements (Persinger, Lafrenière and Ossenkopp, 1974) also have demonstrated significant decreases in activity for some groups of 30 to 60 day old rats that had been exposed perinatally to 10^{-3}T fields; however other experiments in the same series showed no differences between the RMF-exposed and control groups. Neaga and Ababii (1970) found that chicks exposed from 1 to 10 days of development to 2 x 10^{-2}T pulsatile fields also displayed decrements in GOT values up to 45 days of age, but not afterwards. To our knowledge, no other studies concerned with long term blood serum effects of perinatal exposure to ELF magnetic fields have been reported in the literature. The implications of these changes remain to be determined.

Although the blood serum and tissue changes associated with perinatal and adult RMF exposures were small in magnitude, they were different from the profiles associated with either colony room-experimental room or postweaning/pre-adult exposure caging differences. Rats that were exposed to the continuous light and noise of the experimental room displayed significantly lower blood glucose, phosphorous and albumin/globulin ratios as well as less water and food consumption than rats that had been exposed to normal LD 12:12 colony room conditions. Similarly, rats exposed to the different caging conditions displayed alterations in a multitude of blood and tissue measures. In light of these data, the RMF effects should not necessarily be considered low-level displays of some ubiquitous stress.

A most theoretically devastating result of the present study involved the complete absence of perinatal condition by adult condition interactions. We had surmised from anecdotal biometeorological cases and from immunologic models that singular intense exposures to ELF magnetic field stimuli during major organismic development might "sensitize" the organism such that later adult exposures to less intense fields would be associated with enhanced response alterations. Any such field sensitivity enhancement should have been displayed as an interaction term following statistical analyses. However, these results clearly indicate that rats exposed perinatally to supranatural intensities do not display such predilictions to more natural field intensities.

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